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European Journal of Pharmacology 433 (2001) 151–156

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Effect of huperzine A on working memory in reserpine- or yohimbine-treated monkeys

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Received 9 October 2001; accepted 2 November 2001

Abstract

The effect of huperzine A, a reversible and selective acetylcholinesterase inhibitor, on reserpine- or yohimbine-induced spatial working memory deficits in monkeys has been examined using the delayed response task that depends on the integrity of prefrontal cortex. Reserpine (0.1 mg/kg, i.m.) or yohimbine (0.01 mg/kg, i.m.) led to significant impairments in the monkeys' ability to perform the delayed response task. Huperzine A (0.01 mg/kg, i.m. in reserpine-treated monkeys; 0.01–0.1 mg/kg, i.m. in yohimbine-treated monkeys) significantly improved the reserpine- or yohimbine-induced memory impairments. The effect of huperzine A on memory impairments exhibited an inverted U-shaped dose–response pattern. Our data suggest that huperzine A may improve working memory via an adrenergic mechanism.

Keywords: Catecholamine; Huperzine A; Working memory; Reserpine; Yohimbine; (Monkey)

1. Introduction

Huperzine A, a novel *Lycopodium* alkaloid that is chemically unique in comparison with other agents involved in studies of Alzheimer's disease (Liu et al., 1986), is a reversible, potent and selective acetylcholinesterase inhibitor (Tang et al., 1989). Huperzine A has been found to be an effective cognition enhancer in several animal models such as the escape task of water maze, passive foot-shock avoidance (Lu et al., 1988), spatial discrimination of radial arm maze in rodents (Tang and Han, 1999) and delayed response performance in monkeys (Ye et al., 1999). Clinical studies have demonstrated that huperzine A treatment resulted in significant improvements in the memory of aged subjects and Alzheimer's disease patients without any severe side effects (Xu et al., 1995, 1999; Zhang et al., 1991). Compared to physostigmine, E2020, tacrine and galanthamine, Huperzine A has better therapeutic indices, and peripheral cholinergic side effects are minimal at therapeutic doses (Tang, 1996; Wang and Tang, 1998).

Previous studies in scopolamine-treated rats and monkeys showed that huperzine A improved performance through a cholinergic mechanism in a variety of memory tasks (Tang and Han, 1999), such as Y-maze, the radial-arm maze (Xiong and Tang, 1995, Cheng et al., 1996) and delayed response task (Ye et al., 1999).

In addition to cholinergic alterations, the pathophysiological changes of central catecholaminergic pathways were also reported to contribute to the memory loss in animal models and Alzheimer's disease patients (Davies and Maloney, 1976; Coyle et al., 1983; Arnsten and Goldman-Rakic, 1985; Price et al., 1985; Bondareff et al., 1987; Arnsten et al., 1988; Cai et al., 1993; Arnsten and Cai, 1993; Hoogendijk et al., 1999). Studies indicated that norepinephrine modulated the working memory through α_2-adrenoceptors in monkeys and rats (Arnsten and Goldman-Rakic, 1985; Arnsten et al., 1988; Arnsten, 1993, 1998). Catecholamine depleting agent, reserpine, and α_2-adrenoceptor antagonist, yohimbine, could impair the spatial working memory in monkeys, and the memory deficits could be reversed by α_2-adrenoceptor agonists (Arnsten and Goldman-Rakic, 1985; Arnsten et al., 1988; Cai et al., 1993). α_2-Adrenoceptor

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0014-2999/01/$ - see front matter
PII: S0014-2999(01)01500-X

agonists provided effective "replacement therapy" in reserpine- and yohimbine-treated monkeys.

It was reported that huperzine A could significantly increase acetylcholine release as well as norepinephrine levels following systemic or local administration in the cerebral cortex of rats (Tang et al., 1989; Hanin et al., 1993; Zhu and Giacobini, 1995). We hypothesized that the memory deficits induced by reserpine or yohimbine might be improved by huperzine A via adrenergic mechanisms. The first functional evidence of huperzine A on catecholamine enhancements in the cerebral cortex of monkeys was provided in the present study.

2. Materials and methods

2.1. Subjects

Four adult female rhesus monkeys (#19, #36, #37, #38) were used to examine the effect of huperzine A on reserpine-treated monkeys. The range of age was 5–9 years old. Five adult monkeys (#14, #19, #37, #39, #43) were used to test the effects of huperzine A on yohimbine-treated monkeys. The range of age was 4–9 years old. Monkey #14 was male, and the others were females. Monkey #43 was drug naïve. Although the others had prior experience of drug exposure, none of them had been involved in drug test at least one year preceding the present investigation. All animals were housed with standard laboratory conditions, and normal diets were given immediately after cognitive testing. Daily supplements of fruits were also given, and water was available for all day. The animal experimental protocols that are compatible with animal rights were approved by the Local Committee on Animal Care of Chinese Academy of Sciences.

2.2. Cognitive testing

Cognitive testing was performed in a Wisconsin General Test Apparatus situated in a quiet room. Animals were tested at the same time of day immediately prior to feeding.

The monkeys had been previously trained on the two-well spatial delayed response task as previously described (Cai et al., 1993). The test tray contained a left and a right foodwells spaced 15 cm apart. An opaque screen could be lowered to separate the animal from the test tray for a specified delay. During delayed response, the monkey could watch as the food was put into one of the two foodwells. Then, the foodwells were covered with identical cardboard plaques and the opaque screen was lowered between the animal and the test tray for a specified delay. At the end of this delay, the screen was raised and the animal was allowed to choose using its working memory. Reward was quasi-randomly distributed between the left and right wells over the 30 trials that made up a daily test session. During the initial training phase, delays were held constantly during a daily session and were gradually increased from "0"s according to a step-wise procedure over the 1000 trials.

After 1000 trials, the animals were ready for drug testing. In order to observe the effects of drug on memory capacity, the animals were trained on a variable delayed response task in which five different delays were quasi-randomly distributed over the 30 trials that make up the daily test session. Delays were adjusted until the animal exhibited stable performance level of about 27 trials correct out of a possible 30 trials (about 90% correct) to make enough space to show the effects of impairment of reserpine or yohimbine. For example, the range of delays was "0"–8 s (0, 2, 4, 6 and 8 s) for monkey #37. The "0"-s delay consisted of lowering the screen and immediately raising it again. Once performance was demonstrated to be stable at this baseline for more than 2 days, reserpine or yohimbine treatment was initiated.

2.3. Drug administration

Reserpine (Guangdong Medicine Company of China), yohimbine (Sigma) and huperzine A (provided by the Department of Phytochemistry, Shanghai Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences) were dissolved in sterile saline prior to injection. The injection volume was kept constant at 0.1 ml/kg irrespective of doses.

Two salines or reserpine + saline or reserpine + huperzine A were injected i.m. 30 min before delayed response testing. Saline + saline injections were given daily for 9–12 days to demonstrate stable baseline performance in this task. Daily injections of reserpine + saline were initiated following saline + saline injections for 10–12 days. The animal was challenged with intermittent injections of reserpine + huperzine A after the performance had stabilized at a newer and lower baseline of responding (between 19 and 22 trials correct out of 30 trials) for more than 2 days. Huperzine A injections were at least 5 days apart, at least two drug washout days were required. The doses of huperzine A were 0.0001, 0.001, 0.01, 0.1 and 0.5 mg/kg; the dose of reserpine was 0.1 mg/kg. Two salines or saline + yohimbine or yohimbine + huperzine A were injected i.m. 30 min before delayed response testing.

Yohimbine + saline were injected once after the performance of saline-treated monkeys had a stable baseline above 27 trials correct out of a possible 30 trials in this task. Yohimbine + huperzine A were injected i.m. after saline + saline control was back to baseline level (above 27 trials correct out of 30 trials) for more than 2 days from yohimbine + saline control. The doses of huperzine A were 0.0001, 0.001, 0.01, 0.1 and 0.2 mg/kg; the dose of yohimbine was 0.01 mg/kg.

2.4. Statistics

As each monkey's performance on drug was compared to its own performance on matched saline control sessions,

repeated measures designs were utilized: paired *t*-test (T_{dep}) or one-way analysis of variance with repeated measures followed by Tukey's test as post-hoc analysis.

3. Results

3.1. Effects of reserpine on delayed response performance

Repeated daily injections with salines for 9–12 days had no effect on delayed response testing. Performance remained about 27 trials correct out of 30 trials, and there was little variation in performances over time ($F(4,19) = 1.765$, $P = 0.2008$, no significant change in performance over time). The usual testing time for a single session made up of 30 trials was continuous and lasted for about 0.5 h.

In contrast, repeated daily injection of reserpine for 10–12 days resulted in significant deficits in delayed response performance in all the animals (Fig. 1). Performance dropped from a mean of 26.58 ± 0.01 trials correct on saline to a mean of 21.33 ± 0.20 trials correct on reserpine treatment ($T_{dep} = 11.71$, $P < 0.001$). Reserpine had its maximal effects on performance following the two longest delays, no significant effect at "0"-s delays ($F(1,3) = 2.889$, $P > 0.05$) (Fig. 2). Some signs of the side effects of reserpine, such as the hypotensive, and sedative effects were observed. After repeated daily injection of reserpine for 5–6 days, the tester had to wait until the animal came to do the delayed response task. The animals could not perform the delayed response task continuously over 30 trials, as the inter-trial internal was longer than usual, but they could perform a single trial as usual, therefore the sedation did not affect the task.

3.2. Effects of huperzine A on working memory in reserpine-treated monkeys

Huperzine A significantly improved the delayed response performance in reserpine-treated monkeys ($F(4,19) = 8.22$, $P = 0.002$) (Fig. 3). The dose–response curve was inverted U-shaped with the maximal improvement at 0.01 mg/kg,

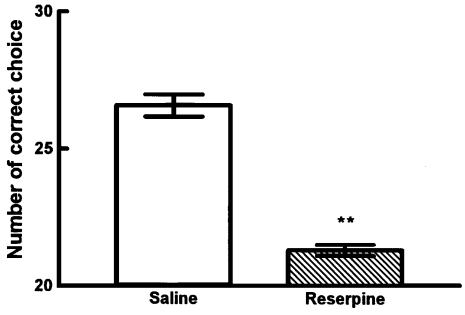

Fig. 1. Effects of repeated saline vs. repeated reserpine (0.1 mg/kg) treatments on the delayed response performance in young adult monkeys ($n = 4$). Saline or reserpine administered i.m. 30 min before testing. Values represent the mean ± S.E.M. number of trials correct out of a possible 30 trials. ** $P < 0.01$ vs. saline control.

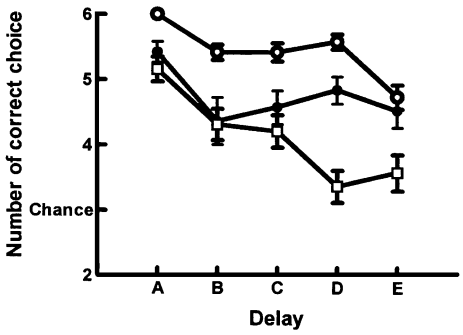

Fig. 2. Effects of saline (○), reserpine (□, 0.1 mg/kg) and huperzine A (●, 0.0001–0.5 mg/kg) on five delay intervals (A, B, C, D and E) used in each testing session in monkeys ($N = 4$). The A delay was always 0 s; the B, C, D, and E delays were incrementally increasing delays (e.g., 2, 4, 6, and 8 s) individually selected for each animal to produce an overall baseline performance of about 67% correct. Values represent the mean ± S.E.M. number of trials correct out of a possible six trials at each delay interval.

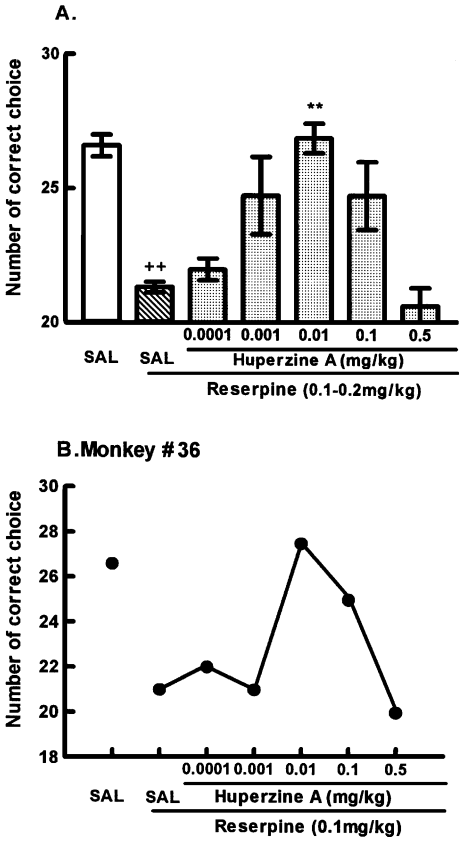

Fig. 3. Effects of huperzine A on the deficit of delayed response performance induced by reserpine (0.1 mg/kg) in young adult monkeys. Saline, reserpine or huperzine A was administered i.m. 30 min before testing. (A) Huperzine A produced a dose-related improvement in the delayed response performance of young monkeys ($n = 4$). Values represent the mean ± S.E.M. number of trials correct out of a possible 30 trials. ++ $P < 0.01$ vs. saline control, ** $P < 0.01$ vs. reserpine control. (B) Effects of huperzine A on the deficit of delayed response performance in monkey #36, one of the four reserpine-treated monkeys. Values represent the mean number of trials correct out of a possible 30 trials.

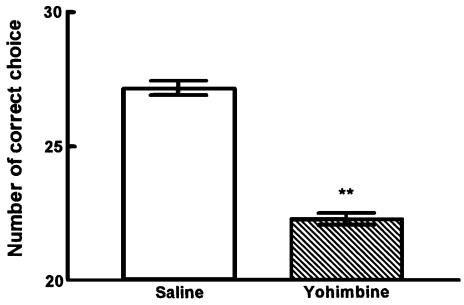

Fig. 4. Effects of repeated saline vs. repeated yohimbine (0.01 mg/kg) treatments on the delayed response performance in young adult monkeys ($n=5$). Saline or yohimbine administered i.m. 30 min before testing. Values represent the mean ± S.E.M. number of trials correct out of a possible 30 trials. $**P<0.01$ vs. saline control.

($F(1,3)=15.70$, P = 0.0011, compared with reserpine control). The trials correct increased from 21.33 ± 0.20/30 to 26.88 ± 0.55/30 after injection of the optimal dose (0.01 mg/kg). The beneficial effects of huperzine A were most evident at the longer delays (Fig. 2). Neither the lowest nor the highest dose had effects ($F(1,3)=1.00$, $P=0.39$ for 0.0001 mg/kg; $F(1,3)=0.09$, $P=0.79$ for 0.5 mg/kg, compared with reserpine control). The side effects of reserpine such as the hypotensive and sedative effects were partially reversed by huperzine A. At the highest dose (0.5 mg/kg) of huperzine A, some cholinergic side effects such as fasciculation and salivation were observed.

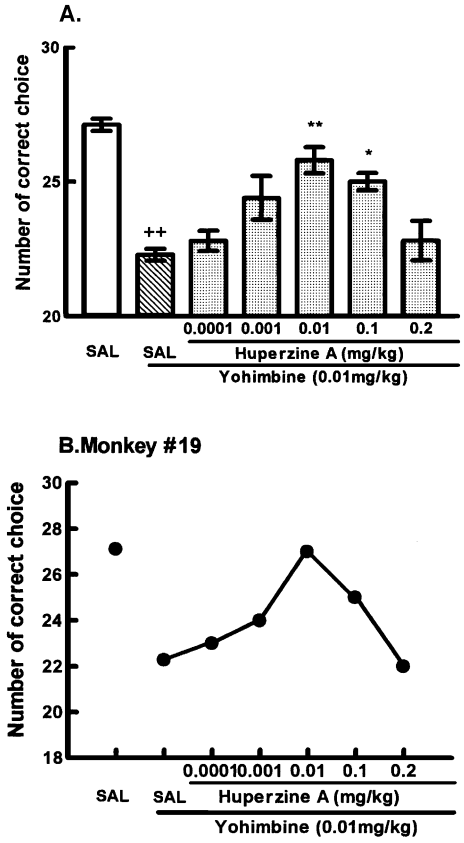

Fig. 5. Effects of huperzine A on the deficit of delayed response performance induced by yohimbine in young adult monkeys. Saline, yohimbine or huperzine A was administered i.m. 30 min before testing. (A) Huperzine A produced a dose-related improvement in the delayed response performance of young monkeys ($n=5$). Values represent the mean ± S.E.M. number of trials correct out of a possible 30 trials. $++P<0.01$ vs. saline control, $**P<0.01$ vs. yohimbine control. (B) Effects of huperzine A on the deficit of delayed response performance in monkey #19, one of the five yohimbine-treated monkeys. Values represent the mean number of trials correct out of a possible 30 trials.

3.3. The effect of yohimbine on delayed response performance

Compared with saline control, yohimbine significantly impaired the delayed response performance at a single dose of 0.01 mg/kg in all monkeys ($F(4,24)=12.47$, $P<0.001$). Performance dropped from a mean of 27.12 ± 0 23 trials correct on saline control to a mean of 22.28 ± 0.22 trials correct on yohimbine ($T_{dep}=15.35$, $P<0.0001$) (Fig. 4). Performance at all retention intervals was impaired but the magnitude of this effect increased as the retention intervals lengthened especially at the longest delays.

3.4. Effect of huperzine A on working memory in yohimbine-treated monkeys

Huperzine A significantly improved the delayed response performance in yohimbine-treated monkeys ($F(4,24)=12.47$, $P<0.0001$) (Fig. 5). The dose–response curve was inverted U-shaped with the maximal improvement at 0.01 mg/kg, the correct trials in performance increased from 22.28 ± 0.22 trials on yohimbine + saline to 25.80 ± 0.49 on yohimbine + huperzine A ($F(4,24)=12.47$, $P<0.001$). Neither the lowest nor the highest dose had effects ($F(4,24)=12.47$, $P>0.05$ for both 0.0001 and 0.2 mg/kg, compared with yohimbine + saline control). The beneficial effect of huperzine A was most evident at the longest delays (Fig. 6).

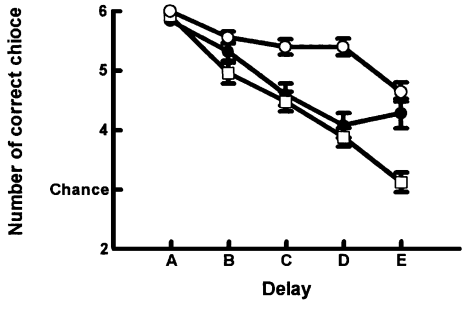

Fig. 6. Effects of saline (○), yohimbine (□, 0.01 mg/kg) and huperzine A (●, 0.0001–0.2 mg/kg) on five delay intervals (A, B, C, D and E) used in each testing session in monkeys ($N=5$). The A delay was always 0 s; the B, C, D, and E delays were incrementally increasing delays (e.g., 2, 4, 6, and 8 s) individually selected for each animal to produce an overall baseline performance of about 67% correct. Values represent the mean ± S.E.M. number of trials correct out of a possible six trials at each delay interval.

4. Discussion

A consistent finding in the present study was that repeated daily injection of catecholamine depleting agent reserpine (0.1 mg/kg) or injection of a single dose of α_2-adrenoceptor antagonist yohimbine (0.01 mg/kg) dramatically impaired the delayed response performance in monkeys via an adrenergic mechanism (Arnsten and Goldman-Rakic, 1985; Cai et al., 1993). Although the shorter delays demanded the same motor and motivational function as the longer delays demand, there was a more significant decrease at longer delays than at shorter delays (Figs. 2 and 6). It means that the major impairments appear to be directly on the memory processes in the prefrontal cortex, since the delayed response task depends on the integrity of the prefrontal cortex (Goldman-Rakic, 1987). Previous studies have indicated that α_2-adrenoceptor agonists, such as clonidine, could improve the memory deficits in reserpine- or yohimbine-treated monkeys (Arnsten and Goldman-Rakic, 1985; Arnsten et al., 1988; Cai et al., 1993), and the effects of clonidine on improving spatial working memory in aged monkeys could not be reversed by α_1-adrenoceptor antagonist, prazosin and β-adrenoceptor antagonist, propranilol but α_2-adrenoceptor antagonist, yohimbine (Arnsten and Goldman-Rakic, 1985; Arnsten et al., 1988). It suggested that the working memory deficits in reserpine- or yohimbine-treated monkeys could be improved by enhancing the endogenous norepinephrine levels of the prefrontal cortex.

In this study, huperzine A significantly improved the performance of reserpine-treated monkeys, and producing an inverted U-shaped dose–response curve. The higher doses of huperzine A even mask the cognitive function of reserpine-treated monkeys via an unknown mechanism. It might be related to the excessive acetylcholine actions, since the cholinergic side effects, such as salivation and fasciculation, were observed after the administration of the higher dose of huperzine A, which was consistent with a previous report (Zhu and Giacobini, 1995).

Huperzine A is a reversible and selective acetylcholinesterase inhibitor. It shows no significant affinity for receptors (Tang et al., 1989), no evident pre- and postsynaptic effects (Lin et al., 1997), the effects of improving spatial working memory of reserpine-treated monkeys are due primarily to the increase of norepinephrine levels from indirect action of huperzine A. It was reported that norepinephrine levels in rats cortex increased significantly following either systemic (i.p.) or local administration in the cerebral cortex of rats with doses of 0.1, 0.3 and 0.5 mg/kg of huperzine A using a microdialysis method; the increase of norepinephrine level was 10 times less than the increase of acetylcholine level by local injection (Zhu and Giacobini, 1995). It was reported that cholinergic agonists could potentiate the release of catecholaminergic neurotransmitters (Weinstock et al., 1979; Xu et al., 1989). The positive clinical effect of some cholinesterase inhibitors such as tacrine has been related to stimulation of both cholinergic and monoaminergic systems (Alhainen et al., 1993). The increase of norepinephrine level may be related to the increase of extracellular acetylcholine level through subcortical mechanism (Zhu and Giacobini, 1995). Cholinergic system interacts with monoaminergic system to control the cognitive function (Riekkinen et al., 1990; Decker and Mcgaugh, 1991).

The results of this study showed that huperzine A improved the spatial working memory deficits induced by yohimbine which was an α_2-adrenoceptor antagonist. It is a powerful evidence to prove that the increased norepinephrine by huperzine A might stimulate α_2-adrenoceptor in the prefrontal cortex to improve the delayed response performance in both reserpine- and yohimbine-treated monkeys. There was a discrepancy at the effective doses of huperzine A on catecholamine system between present behavioral results and previous biochemical results, it may primarily be due to the species difference because our present study happened in monkeys and the previous study happened in rats.

The hypotensive and sedative effects of reserpine were partially reversed by higher doses administration of huperzine A in reserpine-treated monkeys. It supported that the increased norepinephrine induced by huperzine A supplemented the deficits of norepinephrine caused by reserpine. Although the hypotension and sedation made the animal uncomfortable, it did not significantly affect the animal to perform memory task, except it needs more time for the animal to perform a daily session. The testing time for one daily session in reserpine-treated monkeys was longer than usual, as the animals need longer interval between trials to have a rest. The response time for one trial was the same as usual. Our previous study showed that the hypotensive and sedative effects induced by reserpine were exaggerated by α_2-adrenoceptor agonist, clonidine, but clonidine markedly reversed the working memory deficits induced by reserpine. It suggested that the hypotension, sedation and working memory were separately modulated by postsynaptic α_2-adrenoceptors in different brain areas (Cai et al., 1993). It emphasizes that α_2-adrenoceptors play an important role in facilitating the prefrontal cortex cognitive function. According to the above analysis, we consider that the effects of reserpine or huperzine A on hypotension and sedation may not be involved in the memory processes.

Our previous study indicated that the memory deficits induced by muscarinic receptor antagonist, scopolamine, was improved by huperzine A in monkeys (Ye et al., 1999). It suggested that huperzine A improves memory with a cholinergic mechanism. This study has proved that huperzine A enhanced memory with an adrenergic mechanism. Taken together, these results confirm that huperzine A is a promising candidate for clinical evaluation as a treatment for Alzheimer's disease, since Alzheimer's disease patients show multiple neurotransmitter decrease.

Acknowledgement

This work was supported by the National Basic Research Program (G1999054000) of China, National Natural Science Foundation of China (30070251) and Chinese Academy of Sciences (KSCX1-09). The excellent technical assistance of Hua Xian Zhang is gratefully acknowledged.

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